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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/551,876	CONDIE ET AL.	
	Examiner	Art Unit	
	Kevin K. Hill, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 July 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-15,17-25,36-39,46 and 48-60* is/are pending in the application.

4a) Of the above claim(s) *1,3-15,17-25,36-39,52,53,58 and 59* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *46,48-51,54-57 and 60* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

1. Applicant's response to the Requirement for Restriction, filed on July 30, 2007, is acknowledged.

Applicant has elected the invention of Group XI, Claims 46 and 48-60, drawn to a method of stabilizing pluripotent stem cells, the method comprising contacting the pluripotent cell culture with an inhibitor of at least one component of the gamma secretase complex.

Within Group XI, Applicant has elected the Notch inhibitor species "DAPT", an inhibitor of the gamma secretase complex, regarding Claim 60.

In a telephone conversation with Applicant's representative, Kathryn Wade at 404-854-8000, on September 20, 2007, Applicant has elected the pluripotent cell species human embryonic stem cells.

2. Election of Applicant's invention(s) was made with traverse.

Applicant respectfully submits that the common technical feature shared by each of the groups is the use of an inhibitor of the gamma-secretase complex. Applicant also traverses the species election on the basis that the examination of the claims as they relate to each of the claimed inhibitors of Notch signaling would not place a serious burden on the Patent Office because of their close technological relationship and sufficiently small number. MPEP § 803.02. In the alternative, Applicants respectfully submit that the claim should at least be examined with respect to other non-transition state analogs such as compound E, in addition to DAPT. *See* p. 12, para. [040].

Applicants' arguments have been fully considered but are not found persuasive. MPEP §803 states that "If the search and examination of all the claims in an application can be made without serious burden, the Examiner must examine them on the merits, even though they include claims to independent or distinct inventions."

In the instant case a serious burden exists since each limitation, directed to each structurally distinct compound requires a separate, divergent, and non co-extensive search and examination of the patent and non-patent literature. For instance, a search and consideration of the prior art as it relates to III-31-C would not be adequate to uncover prior art related to DAPT.

Further, a search and examination of all the claims directed to all embodiments involves different considerations of novelty, obviousness, written description, and enablement for each claim. In view of these requirements, it is the Examiner's position that searching and examining all of the claims including limitations to the genus of γ-secretase inhibitors in the same application presents a serious burden on the Examiner for the reasons given above and in the previous Restriction Requirement.

Each inhibitor of Notch signaling compound is structurally and biochemically distinct. While each may inhibit γ-secretase, the art recognizes that γ-secretase is a complex of proteins, not all of which have been identified. Furthermore, Applicant has provided no evidence to demonstrate that each compound will cause the same biological effect on a given cell type, such that each compound is essentially equivalent to the others. "Compound E" is not claimed.

It is noted that should Applicant traverse the species election requirement, that Applicant was invited to submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. Applicant has not done so.

The requirement is still deemed proper and is therefore made FINAL.

3. Claims 1, 3-15, 17-25, 36-39, 52-53 and 58-59 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

4. Claims 46, 48-51, 54-57 and 60 are under consideration.

Priority

5. This application is a 371 of PCT/US04/09817, filed March 31, 2004 which claims benefit of a prior-filed parent provisional application 60/459,129, filed on March 31, 2003 and claims benefit of provisional application 60/516,582 filed October 31, 2003 under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged.

Accordingly, the effective priority date of the instant application is granted as March 31, 2003.

Information Disclosure Statement

Applicant has filed an Information Disclosure Statement on December 27, 2005 that has been considered. Citation WO 02/77204 is lined through because a copy of the document not filed with the instant application. The signed and initialed PTO Form 1449 is mailed with this action.

Specification

6. **The disclosure is objected to** because it contains an embedded hyperlink and/or other form of browser-executable code (pg. 34, line 30; pg 35, line 6). Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP §608.01.

Claim Objections

7. **Claims 51 and 54 are objected to because of the following informalities:**

These claims each identify DAPT as an agent/compound that may be used in the claimed invention. However, the claims do not first identify the agent/compound by its complete name prior to using its acronym. The abbreviation should be spelled out in the first appearance of the claims and should be followed by the abbreviation in parentheses, e.g. Epidermal Growth Factor (EGF).

Applicant is advised that should claim 51 be found allowable, claim 54 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Claims 51 and 54 (both dependent on claim 50) recite "wherein the inhibitor comprises DAPT".

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. **Claims 46, 48-51, 54-57 and 60 are rejected under 35 U.S.C. 112, first paragraph** as failing to comply with the enablement requirement. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The breadth of the claim is broad for encompassing cell culture methods applicable to a broad genus of distinctly different pluripotent cell populations obtained from an enormous genus

of metazoan animals, wherein the broad genus of distinctly different pluripotent cell populations have distinctly different physiological requirements.

The claims are also broad for not defining the physiological or phenotypical state that is being "stabilized".

The claims are also broad for encompassing a genus of structurally distinct compounds that possess distinctly different biochemical and mechanistic properties that are to be used alone or in combination for the treatment of pluripotent cells.

The inventive concept of the instant application is that the administration of DAPT, a γ-secretase, at a concentration of 50μM will maintain human embryonic stem cells in an undifferentiated state, at least in short-term cultures.

The Existence of Working Examples and The Amount of Direction Provided by the Inventor

The specification discloses the culture of human BGN1 embryonic stem cells maintained on mouse embryonic fibroblasts (pg 47, [0137]). After a degree of manual selection of cells possessing "good cellular morphology" and manual cell passaging, a small number of colonies presented a phenotype of "compact dome morphology" that was stabilized for more than 20 passages (pg 49, [0142]). Magnetic sorting for SSEA4 expression enriched for the compact dome morphology, wherein said cells express the Oct4, SSEA3, TRA-1-60, TRA-1-81 and Notch-1 markers (pg 49, [0143-0144]).

Enzymatic methods to culture and transfer small clumps of hES cells employ the use of trypsin/EDAT to break hES cells down to an essentially single cells suspension for transfer to fresh dishes (pg 54, [0157]). EDTA exposure can activate the γ-secretase complex in hES cells to cleave Notch (pg 56, [0162-0163]). Notch-1 (non-cleaved) is highly expressed on the surface of morphologically undifferentiated hES cells; whereas, differentiating cells are negative for Notch-1 (pg 7, [022]; pg 8, [025]). However, Oct-4, an indicator of undifferentiated state, is expressed in both EDTA-treated (Notch-activated) and EDTA-untreated (Notch-inactivated) samples (pg 56, [0162]), and thus no strict correlation between Notch activation/inactivation and the undifferentiated state exists.

Human ES cells were grown in 50μM DAPT, a γ-secretase inhibitor, for three days prior to EDTA treatment (pg 56, [0163]). Under such conditions, NICD fragment was not generated in BGN1 cultures.

Applicant has discovered that inhibition of γ-secretase reduces the number of spontaneously differentiated cells in hES cell culture and maintains [stabilizes] their pluripotent phenotype when DAPT is used at a concentration of 50μM (pg 57, [0165]). DAPT treatment of trypsin-passaged cultures returned embryoid bodies having a non-cystic phenotype to embryoid bodies having a cystic phenotype. Therefore, inhibition of gamma-secretase appeared to reduce the number and proportion of differentiating cells in hES cultures, thereby leading to an increase in homogeneity of the cultures (pg 58, [0167-0168]).

At issue for the enablement requirement is that the specification does not disclose the culture conditions to predictably use DAPT to maintain the hES cells in an undifferentiated state. While the specification discloses the passaging of hES cells for more than 40 passages (pg 57, Example 5), the working example does not disclose the DAPT treatment condition variables necessary to achieve the reduced number of spontaneously differentiated cells, such as duration, frequency or application before or after critical steps necessary to passage the cells. The specification also does not disclose a nexus between the culture conditions of hES cells and other pluripotent cell populations known in the art, e.g. hematopoietic stem cells, mesenchymal stem cells and neural stem cells, each of which have their own specific cell culture requirements.

The State of the Prior Art and The Level of One of Ordinary Skill

The claims embrace an enormous genus of pluripotent cell types not defined by the specification. To define a cell as a stem cell, scientists have used four criteria (Verfaillie et al, Hematology Am. Soc. Hematol. Educ. Program: 369-391, 2002; pgs 381-382, joining ¶). First, stem cells undergo multiple, sequential self-renewing cell divisions, a prerequisite for sustaining the population. Second, single stem cell-derived daughter cells differentiate into more than one cell type. Examples include hematopoietic stem cells (HSC) that give rise to all hematopoietic cells; neural stem cells (NSC) that give rise to neurons, astrocytes, and oligodendrocytes; and mesenchymal stem cells (MSC) that differentiate into fibroblasts, osteoblasts, chondroblasts, and

adipocytes. Some adult stem cells may give rise to only a single mature cell type, such as the corneal stem cell. A third criterion is that stem cells functionally repopulate the tissue of origin when transplanted in a damaged recipient, which has been shown extensively for HSC and more recently for liver progenitors and NSC. A final, less well established criterion is that stem cells contribute differentiated progeny *in vivo* even in the absence of tissue damage (Verfaillie et al; pgs 381-382, joining ¶). In the era of "Dolly" it has become clear that the genetic information of a cell can be reprogrammed and that somatic cells can dedifferentiate into pluripotent cells. These findings suggest that dedifferentiation and redifferentiation might be a third explanation for adult stem cell plasticity (Verfaillie et al; pg 383, col. 2, last ¶).

The stem cell is a primitive cell that can either divide to reproduce itself (undergo self-renewal) or give rise to more specialized (differentiated) cells. Stem cells exist in many adult tissues. With very few exceptions, stem cells in fetal or adult tissue are generally thought to give rise to only a limited range of differentiated cell types. Such limitations on the developmental repertoire of adult stem cells, imposed by powerful molecular constraints on gene expression, are critical to maintenance of tissue integrity during normal cell turnover and tissue repair: these controls ensure that, for example, when the skin is cut, skin cells rather than, say, prostatic epithelium, grow into and repair the wound. By contrast, ES cells are derived from an embryonic cell population at a stage prior to its commitment to form particular tissues of the body. Because of their origin, ES cells are by nature more versatile than most of their adult counterparts (Verfaillie et al; pg 374, col. 2, ESC).

The morphology, marker expression, and growth requirements of pluripotent cells derived from the human gonad differ in some ways from those of other primate pluripotent stem cells. As in the mouse, the process of conversion from a primordial germ cell to a cell that can be continuously cultured and that is pluripotent is poorly defined. Only a small fraction of the cells give rise to embryoid bodies containing multiple types of differentiated cells. It may be that the conversion from primordial germ cell to pluripotent stem cell is a type of transdifferentiation process that occurs at lower frequency in human cells compared to mouse cells. Further investigation of the cultures derived from embryonic human gonads is required to determine whether permanent lines of pluripotent stem cells can in fact be derived from them (Verfaillie et al, pg 378, col. 1, ¶2).

Pera et al (J. Cell Science 113: 5-10, 2000) teach that the mouse embryonic stem (ES) cell provides a benchmark for definition of the generic requirements for ES cells. Its key features are these: it is derived from a pluripotent cell population; it is stably diploid and karyotypically normal *in vitro*; it can be propagated indefinitely in the primitive embryonic state; it can differentiate spontaneously into multiple cell types representative of all three embryonic germ layers, both in teratomas after grafting or *in vitro* under appropriate conditions; and it can give rise to any cell type in the body, including germ cells, when allowed to colonise a host blastocyst. The criteria for pluripotency usually include derivation of the stem cell line from a single cloned cell. This experiment eliminates the possibility that several distinct committed multipotential cell types are present in the culture that together account for the variety of differentiated derivatives produced.) Thus far, only mouse EG or ES cells meet these generic criteria. Primate ES cells meet the first three four criteria, but not the last. Numerous other candidate mammalian ES cells have been described over the years in domestic and laboratory species, but only in the mouse have all criteria been met rigorously (Pera et al; pg 6, col. 2, ¶2).

Dissimilarities between Mouse and Human ESCs

Primate pluripotent stem cells are different in many respects to mouse ES cells, particularly in their morphology and their ability to withstand dissociation into single cells, so it is important to recognize the correct cell type and handle it appropriately during subculture (Pera et al; pg 7, col. 1, ¶1). How do the phenotypes of the cells – morphology, antigen expression, growth requirements – compare with one another and with those of other types of pluripotent cells, such as EC cells or mouse ES cells? Human EC cells and monkey and human ES cells have phenotypes which are very similar and readily distinguished from those of their counterparts in the mouse and from those of human EG cells (Pera et al; pg 7, col. 2, last ¶). The primate cells grow in flat colonies with distinct cell borders in monolayer culture whereas mouse ES cells grow in more rounded clumps with indistinct cell borders.

Primate pluripotent cells express SSEA-3 and SSEA-4 (the epitope recognised by the latter is more readily detected than that seen by the former), and express SSEA-1 only upon differentiation. Essentially the reverse is true of mouse ES cells (Pera et al; pg 8, col. 1). The Examiner notes that the claims (claim 56) requires the cells to express SSEA4; however Pera et

al teach that murine stem cells do not express SSEA4 (Pera et al; pg 8, Table 1). Furthermore, self-renewal of mouse ES cells, unlike human ES cells, requires activation of STAT3 (O'Shea, Biol. Reprod. 71: 1755-1765, 2004; pg 1756, col. 2, ¶1).

The morphology, marker expression and growth requirements of pluripotent cells derived from the [primate] gonad differ in some ways from those of other primate pluripotent stem cells. The cells grow in more rounded clumps that lack distinct cell borders and are very difficult to dissociate. They express SSEA-1 in addition to SSEA-3, SSEA-4, and TRA 1-60, and they contain alkaline phosphatase activity (Pera et al; pg 8, col. 2, ¶2). As in the mouse, the process of conversion from a primordial germ cell to a cell that can be continuously cultured and that is pluripotent is poorly defined.

Difficulties in Culturing ESCs to Maintain Undifferentiated State

When grown in suspension culture, ES cells form small aggregates of cells. These aggregates have been termed embryoid bodies "EB" and are often employed as models of differentiation and gene expression in early development (O'Shea; pgs 1759-1760, joining ¶). The aggregates become cystic over the course of several weeks. (Verfaillie et al; pg 379, col. 2, ¶2). The isolation of different stem cells and their initial cultivation until the establishment of a line is very specific for each type of stem cell (Ulloa-Montoya et al, J. Biosci. Bioeng. 100(1): 12-27, 2005; pg 14, col. 2, lines 4-7). The mechanism by which ES cells can be expanded while maintaining their tri-lineage differentiation (three somatic germ layers) has not been elucidated, and are likely subject to variations due to unknown factors produced by feeder cells (Ulloa, pg 18, col. 2, ¶2). It may also be that ES cells have undergone certain changes in gene expression to facilitate their immortalized growth *in vitro* (Verfaillie et al; pg 376, col. 1, lines 1-3).

However, current understanding is far from perfect and several key challenges need to be overcome for the culture of undifferentiated hESCs in a therapeutically relevant manner. A major problem in the design of bioprocesses for hESC propagation is the need for the development of rapid, robust and predictive assays for screening the impact of culture manipulations on hESC maintenance (Rao et al, Curr. Op. Biotechnol. 16: 568-576, 2005; pg 572, col. 2). In the mESC system, the correlation of gene expression profiles using functional assays that assess developmental potential to identify genes that are down-regulated during early differentiation

have been reported. However, in the case of hESCs, similar studies that compare gene expression analysis to functional measures of pluripotency (hence validating a putatively unique signature) have not been reported (pg 573, col. 2).

The adherence of hES cells to each other, although critical in maintaining cell-cell interactions, has presented several challenges in the attempt to passage the cells in a consistent manner and to standardize culture conditions (Hoffman et al, Nature Biotechnol. 23(6): 699-708, 2005; pg 705, col. 1, Passaging). Ideal culture conditions will include a defined matrix, defined media supplemented with recombinant proteins and passaging which allows cell seeding at a consistent cell density. Examination of hES cells over extended periods *in vitro* should be considered an important criterion to demonstrate that hES cell characteristics do not change over time, and that the lines are stable in their expression of markers, expression of telomerase, ability to differentiate and maintenance of a normal karyotype (Hoffman et al; pg 701, col. 1, lines 3-7; pg 704, col. 2, ¶2).

Another point of concern regarding recent finding implicating various signaling families as regulators of self-renewal and pluripotency is the short-term in which the hES cells in some studies were maintained in reported conditions; as such, more stringent protocols must be followed before we can draw any definitive conclusions a to the potential roles of various signaling molecules. Although hES cells have been demonstrated to persist in each of these conditions, it is unclear which, if any, of the culture conditions is the optimal one(s). Regardless, further evaluation and comparison of various reported culture conditions may help to elucidate the signaling pathways required by undifferentiated hES cells, and therefore the optimal culture conditions to maintain them in a pluripotent state (Hoffman et al; pg 705, col. 2, Culture conditions). Although hES cell lines appear phenotypically stable over long-term culture...recent studies provide evidence that various hES cell lines may be in distinct epigenetic or developmental states.

Notch Activity in ES cells

With respect to the requirement of Notch activity as it pertains to the maintenance of an undifferentiated state in hES cells, Walsh et al (Acta Pathological, Microbiologica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003) present a model in which

interactions of the Wnt and Notch signaling pathways are important both in maintaining the undifferentiated state and also in regulating the process of differentiation itself among human EC and ES cells (Walsh et al; pg 206, Fig. 6). Our data suggest that inhibition of Wnt and activation of Notch are features of undifferentiated human EC/ES cells and furthermore, that this situation is transiently reversed shortly after RA treatment. It is possible that the predominance of Notch signaling over Wnt is essential for maintaining the stem cell state and that once the balance is shifted beyond a critical point, the cells embark on a program of differentiation. During differentiation, Notch signaling may also be used and may even play an active role in promoting neurogenesis (Walsh et al; pg 207, col. 1).

Use of DAPT in Cell Culture

The specification discloses that the administration of DAPT will maintain hES cells in an undifferentiated state (pg 5, lines 10-11). DAPT (CAS # 208255-80-5) is an art-recognized γ-secretase inhibitor (Dovey et al, J. Neurochem. 76: 173-181, 2001; *of record in IDS). However, Crawford et al (Developmental Dynamics 236:886-892, 2007) teach that 1μM DAPT enhances neuronal differentiation in embryonic stem cell-derived embryoid bodies, wherein the art recognizes that embryoid bodies comprise pluripotent cell populations. Thus, the enhancement/promotion of differentiation due to exposure of DAPT as taught by Crawford et al is opposite to the phenotype and results achieved by administration of DAPT to pluripotent cells as claimed.

Similarly Gal et al (Biochem. Biophys. Res. Comm.·358:908-913, 2007) teach that glioblastoma stem cells that possess some properties with normal brain stem cells (pg 908, col. 2). Gal et al cultured GMB cells under conditions that favored stem cell growth in an undifferentiated state (pg 909, col. 2, CD133+ stem cells). Exposure to 25μM DAPT disrupted the formation of neurospheres, indicating a loss of "stem cell-like" properties such as self-renewal (pg 911, Figure 2).

The Level of Predictability in the Art

"[I]t is fair to say that the science of pluripotent stem cells is still in its infancy." (Verfillie et al, pg 375, col. 1, lines 12-14). So far, there has been no report of large-scale

expansion of undifferentiated hES cells principally, because the basic biological knowledge of factors that maintain hES pluripotency is still being developed (Ulloa, pg 23, col. 1, ¶2). Considerable progress has been made toward the generation of more defined hES cell culture conditions since initial isolation and growth conditions were described. However, current hES cell culture conditions need to be improved. The optimal culture conditions will be defined by the goals of the investigators and the use of the cells. Although considerable progress has been made toward these goals, there is still a significant challenge ahead (Hoffman et al; pg 706, Future Perspectives). It will be important to identify factors that facilitate growth and inhibit differentiation of human ES cells (Pera et al, pg 9, col. 1, ¶2). Thus, the art recognizes considerable unpredictability in methods of maintaining embryonic stem cells in an undifferentiated state.

The art recognizes a broad genus of biologically and developmentally distinct pluripotent cells, wherein each pluripotent cell type has its own unique cell culture requirements. Significant differences exist between embryonic stem cells between different organisms, e.g. mouse and humans, as well as within organisms, e.g. MSCs, HSCs and NSCs, and no standard cell culture protocol exists to reliably and predictably maintain embryonic stem cells or other pluripotent cells in an undifferentiated state because the cell biological signaling pathways necessary and sufficient to maintain the undifferentiated state are not fully known and understood.

In particular regards to the instant application, the art teaches that Notch activity is required at different times in ES cell culture to either maintain the undifferentiated state or to promote the differentiation of the ES cells. However, the timing and robustness of Notch signaling relative to other important signal transduction pathways is complex and not fully understood. Furthermore, the art teaches that administration of DAPT, which will inherently inhibit Notch activation via inhibition of γ-secretase, induces and/or promotes differentiation of stem cells. These teachings are counter to the requirements of Notch activity in maintaining undifferentiated stem cells and the effective results achieved by the administration of DAPT as disclosed in the instant specification.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Thus, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate that the administration of DAPT to a pluripotent cell culture will stabilize, that is to say, maintain the cells in an undifferentiated state. The art recognizes considerable variance in embryonic stem cell culture protocols and the ability to passage such cell long term while maintaining the cells in an undifferentiated state because said cells regularly spontaneously differentiate. The specification also does not disclose a nexus between the culture conditions of instant hES cells and other pluripotent cell populations known in the art and embraced by the claims, e.g. hematopoietic stem cells, mesenchymal stem cells and neural stem cells, each of which have their own specific cell culture requirements. The instant specification does not disclose the DAPT treatment condition variables necessary to achieve the reduced number of spontaneously differentiated cells, such as duration, frequency or application before or after critical steps necessary to passage the cells. Such specific disclosure is necessary because the art recognizes that Notch signaling, in and of itself as well as in relation to other critical signal transduction pathways, is complex, and teaches a requirement for Notch signaling to maintain the undifferentiated state that is counter to the instant specification. Furthermore, the art teaches that the claimed active method step of administering DAPT promotes differentiation rather than maintaining the undifferentiated state.

Accordingly, the instant claims are rejected for failing to comply with the enablement requirement.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the Applicant regards as his invention.

9. **Claims 46, 48-51, 54-57 and 60 are rejected under 35 U.S.C. 112, second paragraph,** as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The term "stabilize" in claim 46 is a relative term which renders the claim indefinite. The term "stabilize" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The reference values or phenotypes to clearly distinguish a "stabilized" versus "non-stabilized" cells is neither disclosed nor claimed. Dependent claims are included in the basis of the rejection because although they recite and encompass a stabilized pluripotent cell culture, they do not clarify the nature or value of the "stabilizing" state.

Furthermore, the specification discloses the pluripotent cell culture to comprise embryonic stem cells in which some ES cells are undergoing differentiation into pluripotent progenitor cells of different cell types, e.g. neural progenitor cells. Thus, it is unclear which pluritpotent cell type is being stabilized in the claimed method, as well as the corresponding phenotype for the given cell that is being stabilized.

The term "non-transition state analogue" in claim 50 is a relative term which renders the claim indefinite. The term "non-transition state analogue" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The reference state or structure to which "non-transition state" and "analog" refer is neither disclosed nor claimed. Dependent claims are included in the basis of the rejection because although they recite and encompass a stabilized pluripotent cell culture, they do not clarify the nature or value of the "non-transition state analogue" state.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. **Claims 46 and 60 are rejected under 35 U.S.C. 102(b)** as being anticipated by Karanu et al (J. Exp. Med 192(9): 1365-1372, 2000), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001).

Karanu et al teach a method of maintaining the survival and expansion of human hematopoietic stem cells in cell culture, the method comprising the administration of soluble human Jagged-1 extracellular domain (pg 1366, col. 1, hJagged-1 protein; pg 1369, Figure 3), wherein the art recognizes soluble human Jagged-1 extracellular domain to be an inhibitor of Notch signaling (Small et al; pgs 32026-32027, joining ¶).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the Examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the Examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

11. **Claims 46, 48-49 and 60 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (J. Exp. Med 192(9): 1365-1372, 2000), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001) and Walsh et al (Acta Pathological, Microbiologica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003).

Karanu et al teach that the success of *ex vivo* expansion conditions still awaits the identification of factors capable of inducing proliferation of rare stem cells (pg 1366, col. 1), and teach a method of maintaining the survival and expansion of human hematopoietic stem cells in cell culture, the method comprising the administration of soluble human Jagged-1 extracellular domain (pg 1366, col. 1, hJagged-1 protein; pg 1369, Figure 3), wherein the art recognizes soluble human Jagged-1 extracellular domain to be an inhibitor of Notch signaling (Small et al; pgs 32026-32027, joining ¶). Karanu et al teach that the activity of soluble human Jagged-1 extracellular domain provides an opportunity for the optimization for clinical protocols aimed at ex vivo expansion and gene transfer of human stem cells (pg 1366, col. 1).

Karanu et al do not teach the cell culture to be human embryonic stem cells. However, at the time of the invention, Walsh et al taught that Notch molecules are expressed in human embryonic stem cells (pg 204. Figure 5), and that regulation of Notch signaling is important for both maintaining ES stem cells in an undifferentiated state and also directing their differentiation (pg 199, col. 1, ¶2).

It would have been obvious to one of ordinary skill in the art to try administering soluble human Jagged-1 extracellular domain onto human embryonic stem cells so as to maintain the survival and expansion of the cells in an undifferentiated state because "a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipate success, it is likely that product not of innovation but of ordinary skill and common sense." Karanu et al taught that inhibitors of Notch signaling could maintain the survival and expansion of stem cells in an undifferentiated state and Walsh et al taught that embryonic stem cells also express Notch, and thus would likely be responsive to inhibitors of Notch signaling, e.g. soluble human Jagged-1 extracellular domain.

Thus, the invention as a whole is *prima facie* obvious.

12. **Claims 46, 50-51, 54 and 55-57 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (J. Exp. Med 192(9): 1365-1372, 2000), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001) and Walsh et al (Acta Pathological, Microbiologica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003), as applied to claims 46, 48-49 and 60 above, and in further view of Dovey et al (J. Neurochem. 76: 173-181, 2001; *of record in IDS), as evidenced by Pera et al (J. Cell Science 113: 5-10, 2000) and Nakhei et al (Nucleic Acids Res. 26(2): 497-504, 1998).

The prior cited art does not teach the use of the γ-secretase inhibitor DAPT as to maintain the survival and expansion of stem cells in an undifferentiated state. However, at the time of the invention, Dovey et al taught that DAPT is an inhibitor of γ-secretase and would likely [negatively] affect the cleavage of other γ-secretase targets, such as Notch (pg 179, col. 1, last ¶).

Dovey et al do not teach that DAPT would stabilize a pluripotent state for at least 10 passages (claim 55), wherein the pluripotent state is determined by expression of SSEA4 and Notch1 in at least approximately 60% of the cells (claim 56) and/or wherein less than approximately 20% of the cells express HNF4α after approximately 10 passages (claim 57). However, absent evidence to the contrary, the limitations of claims 55-57 are necessarily inherent features of a embryonic stem cell culture passaged in the presence of an inhibitor of Notch signaling, specifically DAPT. The prior art recognized that human embryonic stem cells express Notch 1 (Walsh et al) and SSEA-4 (Pera et al; pg 8, Table 1), and that HNF4α is a tissue-specific transcription factor mainly expressed in endodermal tissues such as liver, kidney, intestine and endocrine pancreas, thus indicating differentiation of stem cells towards endodermal tissues (Nakhei et al). Thus, a human embryonic stem cell population passaged in the presence of an inhibitor of Notch signaling, specifically DAPT, will necessarily express the recited markers in the recited proportions for the recited number of passages because an inhibitor of Notch signaling is expected to maintain the survival and expansion of stem cells in an undifferentiated state.

It would have been obvious to one of ordinary skill in the art to substitute soluble human Jagged-1 extracellular domain as taught by Karanu et al with DAPT taught by Dovey et al with a reasonable chance of success because the simple substitution of one known inhibitor of Notch

signaling for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

Thus, the invention as a whole is *prima facie* obvious.

Conclusion

13. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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